

CLAIMS

What is Claimed:

1. A plastid transformation vector for stably transforming a plastid genome comprising, as operably linked components, a first flanking sequence, a DNA sequence coding for *psbA* or a substantially homologous sequence of *psbA*, a 5' untranslated region (UTR), a DNA sequence coding for human serum albumin (HSA), and a second flanking sequence.
 2. The vector of Claim 1 further comprising a regulatory sequence.
 3. The vector of Claim 2, wherein said regulatory sequence comprises a promoter operative in said plastid.
 4. The vector of Claim 2, wherein said promoter is *Prrn*.
 5. The vector of Claim 1, wherein the transformation vector is competent for stably integrating in the plastid genome of higher plant species and wherein the flanking sequences are substantially homologous to sequences in a spacer region of said plastid genome, and wherein said flanking sequences are conserved in the plastid genome of said higher plant species.
 6. The vector of Claim 5, wherein said spacer region is a transcriptionally active spacer region.
 7. The vector of Claim 1, wherein the plastid is selected from the group consisting of chloroplast, chromoplast, amyloplast, proplastide, leucoplast and etioplast.
 8. The vector of Claim 1, further comprising a DNA sequence encoding a selectable marker.
 9. The vector of Claim 8, wherein said selectable marker is an antibiotic-free selectable marker.
 10. The vector of Claim 9, wherein said DNA sequence encoding a selectable marker encodes Betaine aldehyde dehydrogenase (BADH).
 11. The vector of Claim 8, wherein said DNA sequence encoding a selectable marker encodes an antibiotic resistance selectable marker.
 12. The vector of Claim 11, wherein said antibiotic resistance selectable marker is *aadA*.
 13. The vector of Claim 1, wherein the 5' UTR is a 5' UTR of said *psbA*.

14. A plastid transformation vector for stably transforming a plastid comprising, as operably linked components, a first flanking sequence, a promoter operative in said plastid, a DNA sequence coding for a selectable marker capable of expression in said plastid, a DNA sequence coding for human serum albumin or a 5 substantially homologous sequence thereof, and a second flanking sequence.

15. The vector of Claim 1, competent for hyper-expression of HSA, in a plant transformed with said plastid transformation vector, wherein said plant expresses at least 0.1 mg HSA per gram/g fresh weight of said plant.

16. An isolated HSA, wherein said HSA is contained within inclusion 10 bodies, and wherein said inclusion bodies are located in chloroplasts transformed with the vector of Claim 1.

17. The isolated HSA of Claim 16, wherein said inclusion bodies reduce the protolysis of HSA.

18. The isolated and purified HSA of Claim 17, wherein said HSA is 15 recovered from said inclusion bodies wherein said HSA maintains proper folding after the HSA is recovered from said inclusion bodies.

19. The isolated HSA of Claim 18, wherein the HSA is properly refolded when removed from said inclusion bodies so that the HSA is structurally equivalent to native human HSA.

20. The isolated HSA of Claim 19, wherein the inclusion bodies facilitate purification of said HSA from other cellular proteins.

21. A plant stably transformed with the transformation vector of Claim 1.

22. A progeny of the plant of Claim 21.

23. A seed of the plant of Claim 21.

25. 24. A part of the plant of Claim 21, comprising a chloroplast including said DNA sequence coding for human serum albumin (HSA).

25. The plant of Claim 21, wherein said plant further comprises at least one chloroplast transformed with the vector of Claim 1.

30. 26. The chloroplast of Claim 25, wherein said chloroplast contains at least one HSA inclusion body.

27. The plant of Claim 21, wherein said plant further comprises mature leaves transformed with the vector of Claim 1.

28. The plant of Claim 21, wherein said plant further comprises young leaves transformed with the vector of Claim 1.

29. The plant of Claim 21, wherein said plant further comprises old leaves transformed with the vector of Claim 1.

5 30. The plant of Claim 21, wherein the expression of HSA in said plant is at least 0.1 mg HSA/g fresh weight of the plant.

31. The plant of Claim 21, wherein said expression of HSA is about 0.25 mg HSA/g fresh weight of the plant.

32. A method for producing Human Serum Albumin (HSA) comprising:

10 integrating a plastid transformation vector according to Claim 1 into the plastid genome of a plant cell; and

growing said plant cell to express said HSA.

33. The method of Claim 32, further comprising:

isolating HSA inclusion bodies from said plant cell; and

15 extracting HSA from said HSA inclusion bodies.

34. The method of Claim 33, wherein extracting said HSA from said HSA inclusion body is performed by solubilizing said HSA inclusion body with an appropriate solution capable for the solubilizing said HSA inclusion body.

20 35. The vector of Claim 1, wherein said DNA sequence coding for the human serum albumin (HSA) is located in an inverted repeat region of said plastid genome.

36. The vector of Claim 1, wherein said DNA sequence coding for the human serum albumin (HSA) is located in a single copy region of said plastid genome.

25 37. The vector of Claim 1, wherein said DNA sequence coding for the human serum albumin (HSA) is regulated by plastid 5' and 3' elements.

38. The vector of Claim 37, wherein said plastid 5' and 3' elements are 5' and 3' elements of *psbA*.

39. A plant cell comprising a plastid stably transformed with the vector of Claim 1.

30 40. A method of hyper-expressing a biopharmaceutical protein of interest comprising:

obtaining a plant plastid,

transforming said plastid with an expression vector comprising a nucleic acid that encodes said biopharmaceutical protein of interest and, wherein said vector further comprises, as an operably linked component *psbA5'UTR*, wherein said *psbA5'UTR* is located upstream of said biopharmaceutical protein of interest,

5 expressing said biopharmaceutical protein in said plastid, and
recovering said biopharmaceutical protein of interest.

41. The method of Claim 40, further comprising exposing a sample of leaves containing said plant plastid to between 8 and 50 hours of continuous illumination.

10 42. The method of Claim 40, further comprising exposing a sample of leaves containing said plant plastid to 50 hours of continuous illumination.

43. The method of Claim 40, further comprising exposing a sample of leaves containing said plant plastid to 16 hours of continuous illumination.

44. A plastid transformation vector for stably transforming a plastid genome
15 comprising, as operably linked components, a first flanking sequence, a DNA sequence
coding for human serum albumin (HSA), and a second flanking sequence.